

Photoresponsiveness of Turkey Breeder Hens Changes During the Egg-Laying Season: Relative and Absolute Photorefractoriness¹

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ABSTRACT Photosensitive species undergo neuroendocrine changes during a reproductive season that cause them to gradually become unresponsive to a photoperiod that initially stimulated reproduction. They may first become relatively photorefractory (rPR), when they will cease egg laying only if photoperiod is reduced, and then absolutely photorefractory (aPR), when they will cease laying despite long day length. Our objective was to test the photoresponsiveness of breeder turkey hens during egg production at various times following photostimulation and to relate photoresponsiveness to rPR and aPR as well as plasma levels of prolactin (PRL) and luteinizing hormone (LH). Hens were maintained in cages in light-controlled facilities and photostimulated at 31 wk of age (September) with a photoperiod of 16L:8D. At 8, 14, and 20 wk after photostimulation, treated hens received a 2-wk exposure to an 11.5L:12.5D photoperiod and were then returned to 16L:8D. Exposure to the shortened photoperiod at 8 wk of photostimulation resulted in three

distinct responses of declining egg production: nonresponders (NR, 32.7% of hens), partial responders (PAR, 43.9%), or full responders (FR, 23.4%). Egg production returned to control levels following return to a 16L:8D photoperiod. This response repeated at the 14- and 20-wk treatment periods but with greater declines in egg production in the NR and PAR groups. The incidence of subsequent aPR in the NR, PAR, and FR groups was 5.7, 8.5 and 24%, respectively, as compared to 23.3% for the controls. Plasma LH and PRL concentrations also declined in response to 11.5L:12.5D and also rebounded following return to 16L:8D. The hormonal responses of NR, PAR, and FR were similar. We conclude that turkey hens exhibit varying degrees of rPR early during the egg laying season and that the incidence and severity of the rPR response increases as the laying season progresses. Further, PRL and LH levels did not reflect the differences in egg production among the responder and nonresponder groups to changes in photoperiod.

(*Key words:* turkey, photorefractoriness, prolactin, photoresponsiveness, luteinizing hormone)

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INTRODUCTION

Typically, photosensitive avian species undergo neuroendocrine changes during a reproductive season that cause them to gradually become unresponsive to a photoperiod that initially stimulated reproduction. They may first become relatively photorefractory (rPR), when they will cease laying only if photoperiod is reduced, and then absolutely photorefractory (aPR), when they will cease laying despite long day length. That rPR is a lesser form of and precedes aPR has been suggested in several reports (Follett et al., 1984; Nicholls et al., 1988; Bentley et al.,

1997). The development of photorefractoriness is a natural process that assures that reproductive activity will occur at times of the year that maximize chances for survival of the young in nature (Nicholls et al., 1988).

Turkey breeder hens become both rPR and aPR (Siopes, 2001; Proudman and Siopes, 2002). Egg laying by turkey hens is therefore dependent on the dynamic interchange occurring between the physiological states of photosensitivity and photorefractoriness. Because there is an inverse relationship between PR and egg production, the presence of PR can have considerable adverse effects on reproductive performance and breeder economics.

With typical turkey breeder hen management, a hen is maximally photosensitive following an appropriate light-restriction period. She can then respond to long day lengths with typical onset and peak egg production and

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Abbreviation Key: aPR = absolute photorefractoriness; FR = full responder; LH = luteinizing hormone; NR = nonresponder; PAR = partial responder; PRL = prolactin; rPR = relative photorefractoriness.

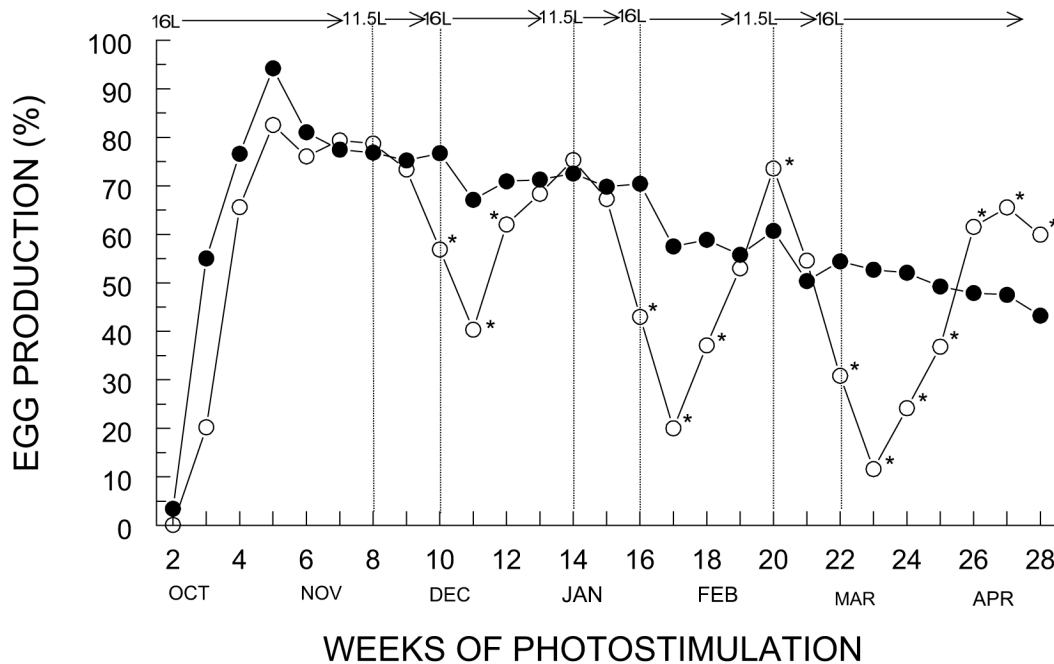


FIGURE 1. Percentage hen-day egg production for hens receiving a reduction in photoperiod from 16L:8D to 11.5L:12.5D for 2 wk starting at 8, 14, and 20 wk of photostimulation and then returned to 16L:8D (photoperiods indicated at top of graph). Vertical dashed lines represent the start and finish of the 2-wk reduced photoperiod treatment. ● = controls, $n = 116$; ○ = treated hens, $n = 107$. *Significantly different ($P \leq 0.05$) from controls within the same time period. Controls remained on 16L:8D throughout. The decline in egg production was 49, 73, and 84% at the 8-, 14-, and 20-wk treatment periods, respectively.

may be considered to have maximum photoperiodic drive and minimum photorefractoriness. As the laying season progresses, photosensitivity and photoperiodic drive are overcome by photorefractoriness as described by Siopes (2001, 2002). In this process rPR is present but not sufficiently strong to overcome photoperiodic stimulation (drive) and stop egg production. However, if the photoperiodic drive is reduced sufficiently, such as by changing from a long day to a shorter but still egg-promoting day length, the inhibitory effects of rPR can predominate, and egg production decreases or ceases. Since the inhibitory inputs of rPR are relatively weak, a subsequent increase in photoperiod may then overcome the inhibition, and the hen will return to egg laying. This latter response distinguishes between rPR and aPR nonlaying hens. The balance between photoperiodic drive and photorefractoriness has been described for chickens and other birds by Sharp (1993, 1996) and for turkeys by Siopes (2001) and Proudman and Siopes (2002).

Clearly, photoresponsiveness of a flock of turkey hens changes during the laying season as more and more hens become aPR. Siopes (2001, 2002) reported the mean onset of aPR was about 17 to 24 wk of photostimulation depending on season and occurred within a range of 7 to 32 wk of photostimulation. However, the temporal aspects of rPR during the lay season have not been established. Our objective was to test the photoresponsiveness

of breeder hens during egg production at various times following photostimulation and to relate photoresponsiveness to rPR and aPR, as well as plasma levels of prolactin (PRL) and luteinizing hormone (LH).

MATERIALS AND METHODS

Female parent line B.U.T.A.³ strain 37 roaster turkeys were raised from day-old following the guidelines of the primary breeder. Birds were raised on 14 h of light per day (14L:10D) until 18 wk of age and then on 6 h of light per day (6L:18D) until 31 wk of age. Hens were moved at 31 wk of age (September 27) to individual cages in two light-controlled rooms and photostimulated with daily photoperiods of 16L:8D (lights on at 0500 h). One room consisted of 116 control hens and the other of 107 treated hens. All lighting was from cool white fluorescent lamps, which delivered a mean intensity of 263 lx at turkey head height. Feed and fresh water were provided for intake ad libitum throughout the study.

The control group received an unchanging photoperiod of 16L:8D throughout the 28-wk study. At 8 (November), 14 (December), and 20 wk (February) after photostimulation, the treated hens received a 2-wk exposure to an 11.5L:12.5D photoperiod by reducing the off-time of the existing 16L:8D photoperiod. After 2 wk, they were then returned to 16L:8D. The ability of a marginal long day length to maintain egg production in turkey breeder hens is a useful tool to assess photoresponsiveness. Siopes (1994) reported that 11.5 h of light per day just exceeded the critical day length for winter egg production by tur-

³British United Turkeys of America, Lewisburg, WV.

TABLE 1. Statistical results for the egg production response illustrated in Figure 2¹

Treatment	Weeks photostimulation																											
	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28							
Control	a	a	a	a	a	a	a	a	a	a	a	a	a	a,c	a	a	a	a	a	a	a							
No response (NR)	a	a	a	a	c	a	a	a	c	c	a,d	a	b	a,c	c	c	c	a	b	b	b							
Partial response	a	a	c	c	a	a	a	a	c	c	c,d	a	b	c	c	c	c	c	b,c	b	b							
Full response	a	a	b	b	b	b	a	a	b	b	b	b	a,b	b	b	b	b	b	a,c	b	a,c							

¹Treatments with common letters within a week of photostimulation are not significantly ($P \leq 0.05$) different.

keys and therefore would qualify as a marginal long day length. That a laying hen reduces or ceases egg production in response to a change from longer photoperiods to 11.5 h of light per day indicates some presence of photorefractoriness. If egg production resumes on return to longer day lengths, the hen is considered to have been rPR rather than aPR.

Individual hen egg production was recorded daily. Photorefractoriness was determined as described in previous publications (Siopes, 2001, 2002; Proudman and Siopes, 2002), time of onset was recorded, and percentage incidence was calculated. Blood samples were collected from control and treated hens at the start and end of each 11.5 L photoperiod. Five-milliliter blood samples were collected into heparinized tubes from the ulnar vein in the morning (0700 to 1100 h) and plasma was separated and stored at -70°C until assayed for hormone levels.

Based on the degree of decline in egg production response to the light treatments at 8 wk of photostimulation, we formed three distinct treatment response groups: non-

responders (NR), partial responders (PAR), and full responders (FR). The response of hens in each of these groups was then recorded for a repeat of the light treatment at 14 and 20 wk of photostimulation. Each response group was based on egg production during the 2-wk periods immediately before and after the 11.5L light treatment at 8 wk of photostimulation. Nonresponder hens were not different from controls in their egg production. Partial responders had a decline in posttreatment egg production exceeding one standard error of the control levels. Full responders ceased egg production for at least 7 d following the light treatment.

Hormone levels were measured for a minimum of 10 randomly selected hens in the control group and each of the treatment groups. Plasma levels of LH and PRL were measured by RIA. Prolactin was measured using the homologous RIA of Proudman and Opel (1981), and LH was measured using a chicken LH RIA as described by Bacon and Long (1996). All samples were assayed in a single assay for each hormone, and the intraassay coeffi-

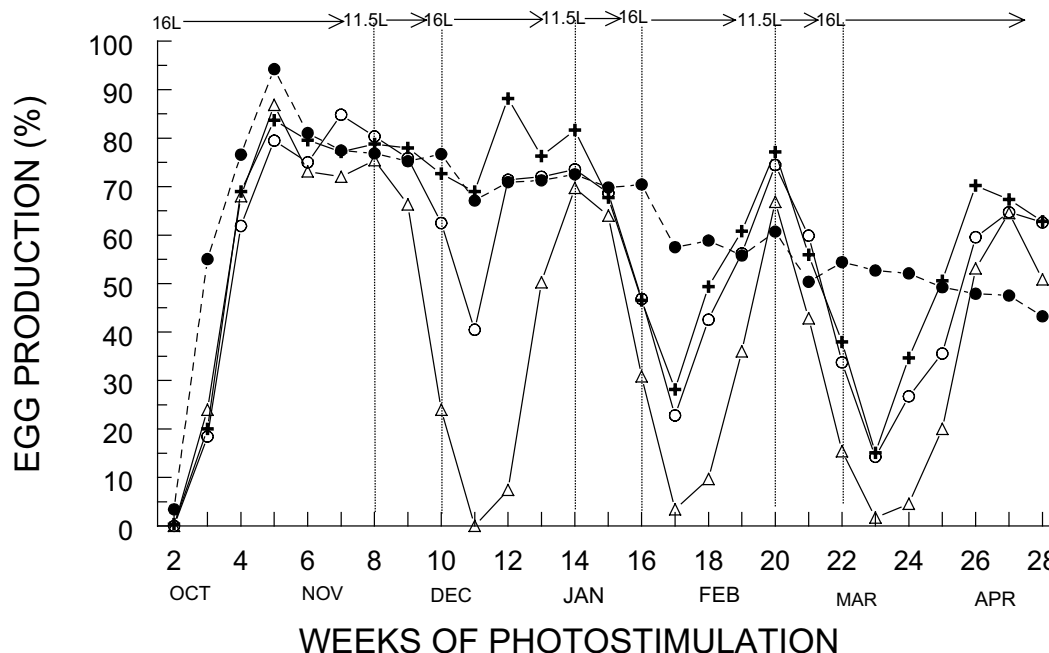


FIGURE 2. Percentage hen-day egg production for subgroups of the treated hens. All treated hens received a reduction in photoperiod from 16L:8D to 11.5L:12.5D for 2 wk starting at 8, 14, and 20 wk of photostimulation and then were returned to 16L:8D. Vertical dashed lines represent the start and finish of the 2-wk reduced photoperiod treatment. Subgroups were based on the degree of decline in egg production in response to reduced photoperiods at the 8-wk treatment period and consisted of + = nonresponders (NR, $n = 35$), \circ = partial responders (PAR, $n = 47$), and \triangle = full responders (FR, $n = 25$). \bullet = controls, $n = 116$. Differences ($P \leq 0.05$) among treatment means within any week of photostimulation are indicated by no common superscripts (and are presented in Table 1 for clarity). Controls remained on 16L:8D throughout.

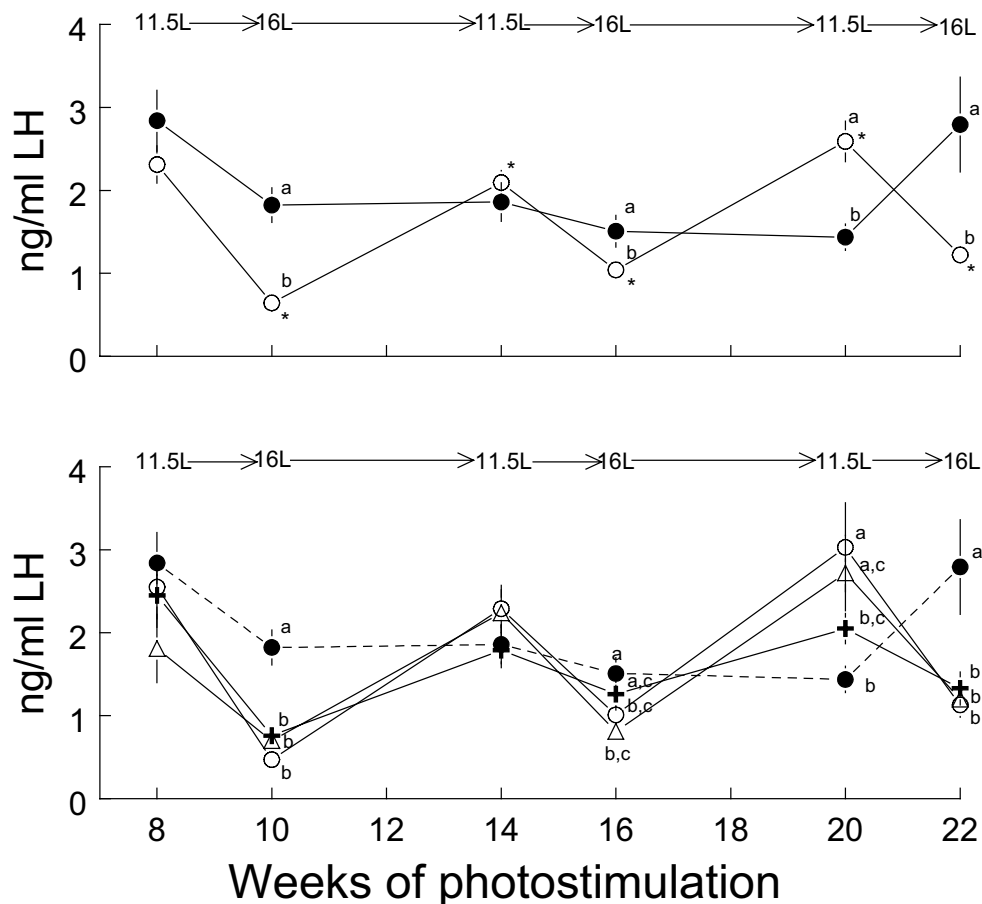


FIGURE 3. Mean (\pm SEM) plasma luteinizing hormone (LH) levels of hens at the start and end of each of the reduced photoperiod (11.5L:12.5D) treatments. Photoperiod changes indicated along the top; controls were on 16L:8D throughout. Top: control (\bullet , $n = 14$) vs. all treated (\circ , $n = 34$) hens; bottom: control (\bullet , $n = 14$) vs. response subgroups as per Figure 2 (Δ , nonresponders, $n = 12$; \circ , partial responders, $n = 12$; $+$, full responders, $n = 10$). Asterisk indicates a significant difference from the preceding value within the same treatment group. Preceding values for each of the subgroup treatment means (bottom graph) were all significantly different, asterisk not shown. ^{a-c}Means with no common letters differ significantly ($P \leq 0.05$) within a period.

cients of variation were 5.7% and 11.9% for PRL and LH, respectively. One-way analysis of variance was used to evaluate treatment effects using the general linear models procedures of the SAS Institute (1990). The least squares means option was used to estimate significant differences among treatment means. From the same statistical source the paired *t*-test was also used to evaluate changes in hormone levels during the 2-wk treatment periods. In addition, chi-square was used to estimate differences in percentage incidence data. Statements of statistical significance are based on $P \leq 0.05$ unless specified otherwise.

RESULTS

Figure 1 presents the mean egg production response of turkey hens on a photoperiod of 16L:8D to sequential 2-wk interruptions with an 11.5L:12.5D photoperiod after 8, 14, and 20 wk of photostimulation. Clearly, the response was not the same at each treatment period, and the effect of each photoperiod change on egg production increased as the laying season progressed. The maximum drop in hen-day egg production was about 49, 73, and 84% for the 11.5L:12.5D treatment at 8, 14, and 20 wk of photostim-

ulation, respectively. After each treatment period, there was a robust return of egg production to at least the level of the untreated control group by 4 wk posttreatment and return of the 16L:8D photoperiod. Figure 2 and Table 1 present the egg production responses by hens to reduced day length and show that individual responses varied to include NR, PAR, FR as early as 8 wk after photostimulation (peak production occurred at 5 wk after photostimulation). These groups were defined in the Materials and Methods section. The percentage distribution of the 107 hens into NR, PAR, and FR was 32.7, 43.9, and 23.4%, respectively. The response of the FR was very similar at each of the three time points examined and was characterized by a short-day-induced cessation of lay followed by a long day resumption of lay at each time period. For the NR and PAR hens the short-day-induced drop in egg production worsened at the 14- and 20-wk testing periods, as compared to the response at 8 wk of photostimulation, but group egg production never ceased. At every testing period, all groups had a robust return of lay to at least the level of the control hens following return of long days. The strength of egg production recovery of the flock after return of long day lengths,

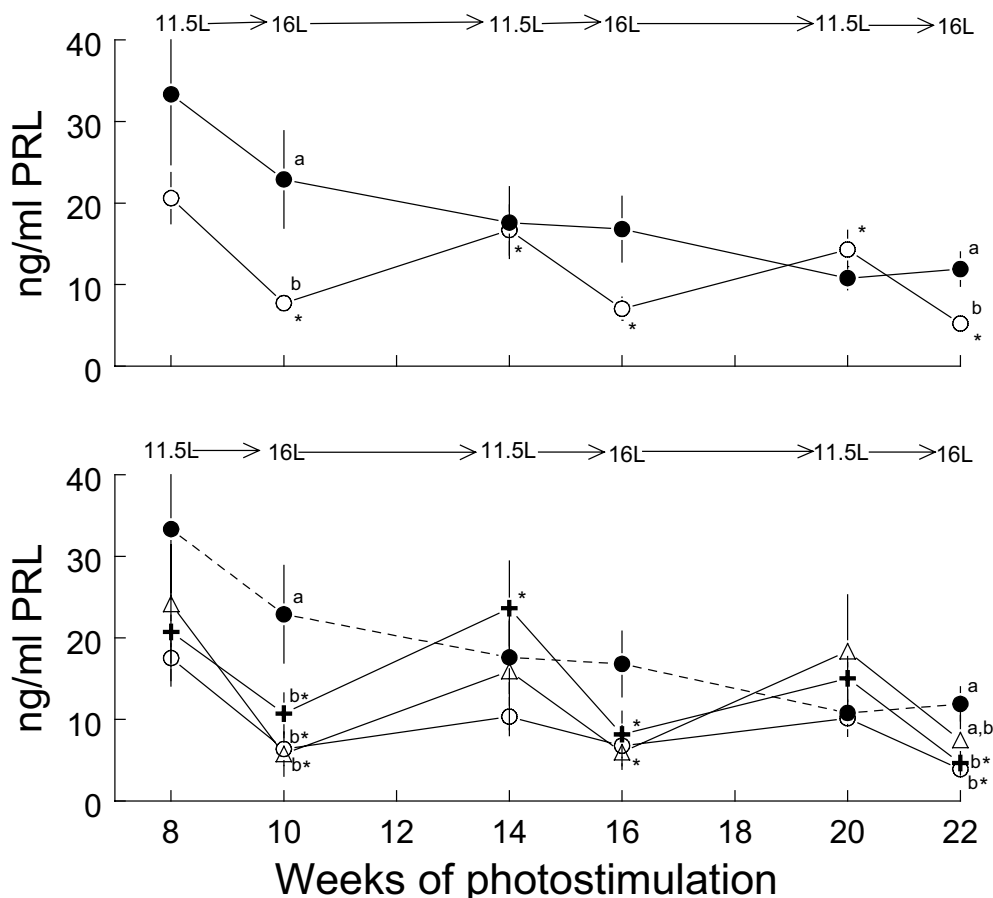


FIGURE 4. Mean (\pm SEM) plasma prolactin levels of hens at the start and end of each of the reduced photoperiod (11.5L:12.5D) treatments. Photoperiod changes indicated along the top; controls were on 16L:8D throughout. Top: control (●, $n = 14$) vs. all treated (○, $n = 34$) hens; bottom: control (●, $n = 14$) vs. response subgroups as per Figure 2 (△, nonresponders, $n = 12$; ○, partial responders, $n = 12$; +, full responders, $n = 10$). Asterisk indicates a significant difference from the preceding value within the same treatment group. ^{a,b}Means with no common letters differ significantly ($P \leq 0.05$) within a time period.

especially late in the lay season, implies a predominance of photosensitive hens, if not an absence of PR hens. However, PR hens were present.

Absolute PR was observed in 23.3% (27/116) of the control hens during the 28-wk study, whereas 11.2% (12/107) of the hens, which received the periodic reductions in photoperiod, became aPR. Less aPR occurred in the NR and PAR groups (5.7% and 8.5%, respectively), while the hens in the treated group that responded fully at 8 wk became aPR at a rate similar to the controls (24%; 6/25).

Plasma levels of both LH and PRL for all treated hens declined with each reduction in photoperiod and returned to that of controls by the time of the next treatment (Figures 3 and 4, top panels). Treatment subgroup responses for plasma LH (Figure 3, bottom) and PRL (Figure 4, bottom) generally followed the response pattern described above and did not show a consistent difference related to their classification into NR, PAR, or FR groups at 8 wk.

That rPR can precede aPR is shown in Figure 5. For only hens that eventually expressed aPR, the overall declining egg production pattern for 28 wk of photostimulation was similar in control and treated hens, as was onset

of aPR. In the control hens the mean onset of aPR occurred after 20.3 ± 0.9 wk of photostimulation and in the treated was 19.8 ± 1.1 wk. In response to the first two 11.5L:12.5D treatments, hens had a partial decline in egg production that fully returned with the return of 16L:8D. Following the last treatment at 20 wk of photostimulation egg production ceased and did not reinitiate upon return of 16L:8D. Every hen in the aPR group of hens in Figure 5 had a decline in egg production, some complete and some partial, in response to reduced photoperiod and prior to becoming aPR.

DISCUSSION

The results of our experiment clearly demonstrate the presence of rPR in a majority of hens by 8 wk after photostimulation, and only 3 wk after the flock reached peak production. That is, egg production declined when the photoperiod was reduced to a marginal long day length (11.5L:12.5D) but returned when the photoperiod was returned to 16L:8D. Hens (FR) that showed the most drastic response to a shorter (but still photostimulatory) photoperiod at 8 wk also showed the most drastic response at subsequent treatment periods. Hens that showed par-

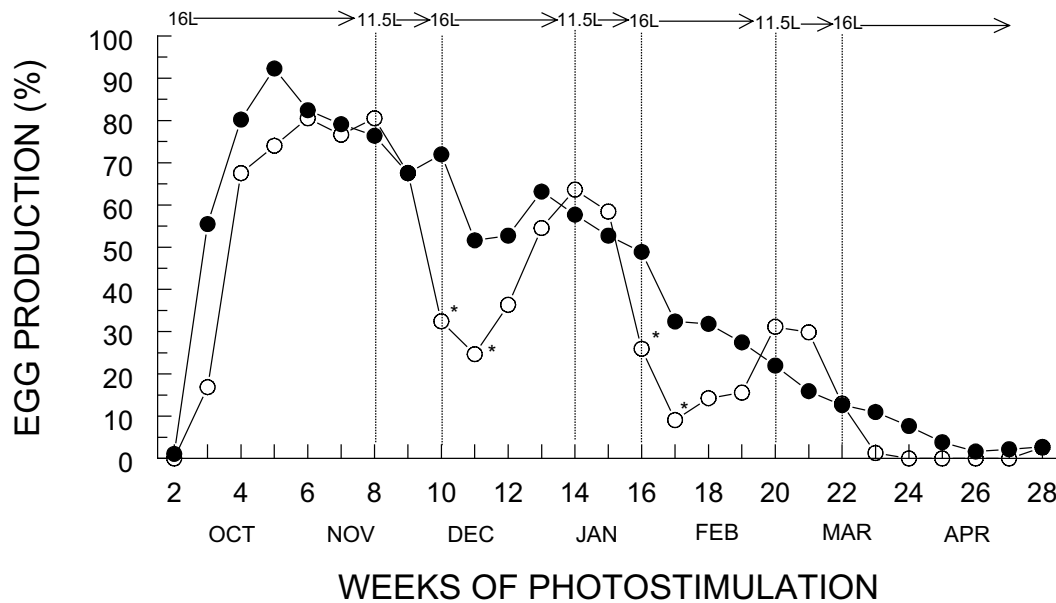


FIGURE 5. Relative photorefractoriness (rPR) precedes absolute photorefractoriness (aPR). Egg production response to shortened day lengths only for hens that expressed aPR during 28 wk of photostimulation. All treated hens (\circ , $n = 12$) received a reduction in photoperiod from 16L:8D to 11.5L:12.5D for 2 wk starting at 8, 14, and 20 wk of photostimulation and then were returned to 16L:8D (indicated across top of graph). Controls that expressed aPR (\bullet , $n = 27$) remained on 16L:8D throughout. Vertical dashed lines represent the start and finish of the 2-wk reduced photoperiod treatment. *Significantly different from controls ($P \leq 0.05$).

tial or no rPR at 8 wk exhibited an increase in their rPR response as the reproductive cycle progressed. This variability in photorefractoriness among turkey breeder hens within and between time periods of the laying season is consistent with our previous report (Siopes, 2001). These results suggest that considerable variability exists among individuals in photoperiodic drive, even early in the production cycle. This was somewhat unexpected for a bird that receives such intense genetic selection. We anticipated the FR group as a normal response and speculate that the PAR and NR groups probably reflect the effects of genetic selection.

Circulating levels of the hormones LH and PRL declined following a reduction in photoperiod and returned to normal levels with the return of long photoperiods, but, somewhat surprisingly, the differences in photorefractoriness among laying hens as determined by changes in egg production were not reflected in circulating levels of either LH or PRL. Nonresponders exhibited as great a decline in basal LH levels following the first exposure to reduced photoperiod as FR hens that ceased laying, indicating that the most photosensitive hens can maintain egg production with quite low levels of circulating LH. However, our data do not preclude the possibility that alterations in the preovulatory surge in LH may account for the observed differences in photorefractoriness.

The incidence of aPR in the controls was 23.3% as compared to 11.2% for all hens receiving a photoperiod reduction and was similar to the FR subgroup (24%) of hens, which received the shorter photoperiod treatment. The lower incidence of aPR seen in the NR (5.7%) and PAR (8.5%) hens suggests that photoperiod treatment may

have reduced the onset of aPR in these groups, thus, contributing to a lowered overall incidence of aPR in treated hens. This could occur if photorefractoriness inputs were low, and the reduced photoperiod was sufficient to dissipate photorefractoriness or retard its development. However, if photorefractoriness was in a more advanced phase, as seen in the FR hens, then a reduced photoperiod should have less effect on the onset of aPR. Additional support for the suggestion of some attenuation of photorefractoriness by reduced photoperiods can be seen in Figures 1 and 2. Egg production rebounded to levels higher than controls following reexposure to 16L:8D, especially for the NR and PAR groups. This response suggests that photoperiodic drive was transiently increased in these groups.

That none of the photorefractoriness subgroups had a complete absence or complete presence of hens that subsequently became aPR suggests that photorefractoriness early in lay (after 8 wk of photostimulation) is not strongly coupled to onset of aPR later in the lay period. However, the observed progressive increase in the rate of expression of aPR across the NR, PAR, and FR subgroups seems logical since a strong response to marginal long photoperiods early in the lay period, such as in the FR group of hens, implies weak photoperiodic drive that could be readily overcome by photorefractory inhibitory processes. Why all hens in the FR group did not subsequently become aPR remains unknown, but clearly photorefractoriness did not develop sufficiently to overcome even weak photoperiodic drive in some hens. The exact opposite argument would apply to the NR subgroup of hens. That is, with such an apparent strong photoperiodic drive to sustain egg production at 8 wk, why did any of these

hens (2/35) become aPR? In the very few that did (2/35), photorefractoriness was able to overcome whatever photoperiodic drive was present. This result suggests that progression from rPR to aPR, or, conversely, loss of photoperiodic drive is not a simple linear progression with time within each individual hen. Otherwise, all hens would become aPR and would do so at about the same time.

Our present study is in agreement with our previous reports that rPR exists in turkey breeder hens (Siopes, 2001; Proudman and Siopes, 2002) and provides strong evidence that rPR is a lesser form of and precedes aPR. This may be seen by the progressive change in photorefractoriness of hens that ultimately became aPR (Figure 5). All hens that became aPR first exhibited rPR during at least one of our testing periods. This conclusion that rPR precedes aPR is in agreement with similar results reported for quail (Follet and Nicholls, 1984), starlings (Bentley, 1997), and tree sparrows (Wilson and Reinert, 1999). How soon rPR first appears in some individuals after photostimulation remains unknown, but our prior studies have shown that aPR can appear as early as 7 wk after photostimulation (Siopes, 2001, 2002). In a practical sense, rPR has reduced importance because as long as hens are maintained on sufficiently long photoperiods the accompanying photoperiodic drive overrides the subtle inhibition associated with rPR, and the hens continue to lay eggs. The most obvious practical problem is an abrupt cessation of lay when rPR progresses to aPR. However, rPR is important because it is part of the process of becoming aPR. In addition, our results suggest that a test for the presence of rPR early in the reproductive cycle may provide a selection tool for identifying breeders with the greatest potential for a long reproductive cycle.

We conclude from these results that photoresponsiveness of domestic turkey hens is heterogeneous both within and between time points during the laying season. Differences in photoresponsiveness were not associated with differences in plasma levels of PRL or LH. In addition,

photoresponsiveness during the laying season is a dynamic process that appears to progress through an initial photosensitive state and then to rPR and aPR physiological states. These states are then associated with initiation of egg production and peak production, spontaneous decline of egg production, and finally, cessation of lay and end of the laying season, respectively.

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